

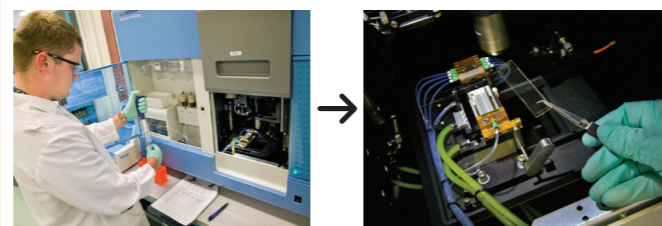
Illumina Sequencing Technology

The Illumina approach

relies on attachment of randomly fragmented genomic DNA to a planar, optically transparent surface and solid phase amplification to create an ultra-high density sequencing flow cell with up to 200 million clusters per slide, each containing ~1,000 copies of template. These templates are sequenced using a four-color DNA sequencing-by-synthesis technology that employs reversible terminators with removable fluorescence. This highly parallel approach yields significant throughput (up to 20 Gigabases, or 20 billion bases per run) with high accuracy. Labeled nucleotides are incorporated at each cycle and high sensitivity fluorescence detection is achieved using laser excitation and total internal reflection optics. Images are compiled and processed to produce base sequences for each DNA template. Short sequence reads (up to 114 bases) are aligned against a reference genome and genetic differences are called using a specially developed data pipeline.

1

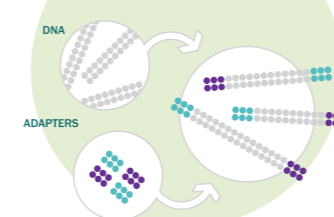
Illumina Instrument



2

Library Construction

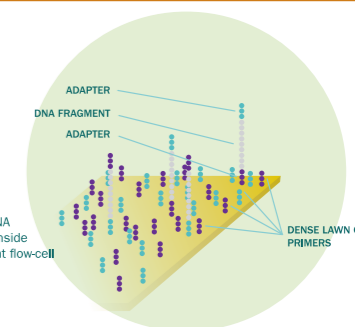
Prepare sample by randomly fragmenting genomic DNA and ligating adapters to both ends of the fragments.



3

Cluster Station—On Chip Hybridization

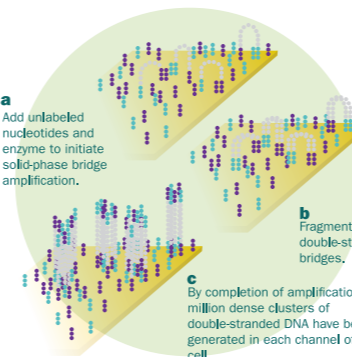
Randomly bind single-stranded DNA fragments to the inside surface of the eight flow-cell channels.



4

DNA Amplification

a Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification.



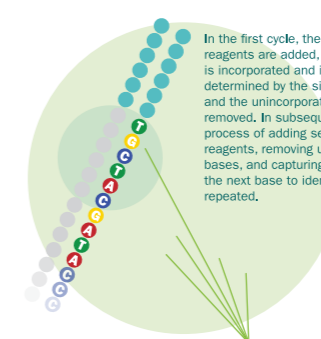
b Fragments become double-stranded DNA bridges.

c By completion of amplification, several million dense clusters of double-stranded DNA have been generated in each channel of the flow cell.

5

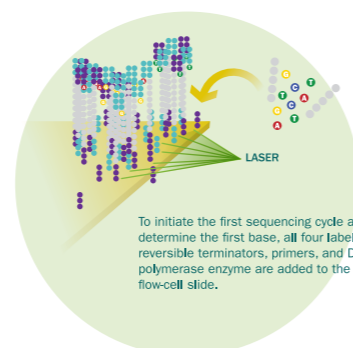
DNA Sequencing

In the first cycle, the sequencing reagents are added, the first base is incorporated and its identity determined by the signal given off, and the unincorporated bases are removed. In subsequent cycles, the process of adding sequencing reagents, removing unincorporated bases, and capturing the signal of the next base to identify is repeated.



6

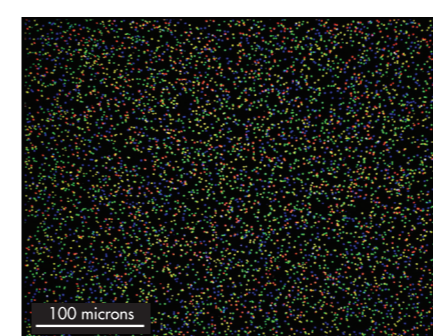
Sequencing-by-Synthesis (SBS)



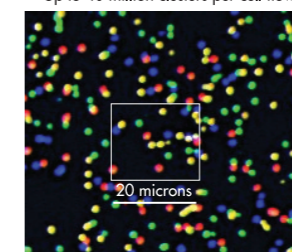
To initiate the first sequencing cycle and determine the first base, all four labeled reversible terminators, primers, and DNA polymerase enzyme are added to the flow-cell slide.

7

Clonal Single Molecule Array™ Technology

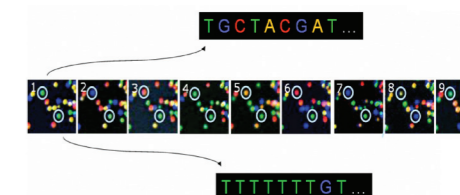


Up to 40 million clusters per cell flow



8

Base-calling from Raw Data



The identity of each base of a cluster is read off from sequential images